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RESEARCH ARTICLE

Studies on degradation of azo dyes with methyl orange as model dye using *Saccharomycers cerevisiae*

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SUMMARY : Baker's yeast as a low price, very high reserve biocatalyst, has been successfully used for the first time to realize total degradation and decolourisation of methyl orange within 24 hours through biological degradation. The degradation performance of yeast for methyl orange under the effect of various factors has been studied. The non-toxic end product characterization has been done with the help of LC-MS, NMR and FT-IR techniques. The outcome of this research shows that baker's yeast S*accharomyces cerevisiae* has satisfactory catalytic effort in degradation of organic compound and the degraded end product is also less toxic to the environment.

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Now-a-days water has been polluted in many ways and one such way is due to the release of unwanted substances to the environment by man in quantities that damage either the health or the resources itself. Rapid growth of industries has increased the water pollution to larger extent. This increase in water pollution has greater impact on environment as it results in the formation of waste waters, gaseous emissions, and solid and semi-solid residues leading to air, ground water, and land pollution followed by degradation. Industries mainly like dye manufacturing, dyeing and textile industries causes a greater deal of environment pollution. There are about 10,000 kinds of dyes used in leather manufacturing and textile industries. Synthetic dyes are extensively used in leather, textiles, paper and printing industries that can be classified as azo, anthaquinone, vat, phthalocyanine, indigo, polymethine, caronium and nitro dyes.

Dyes:

Dyes are used to impart colour to materials of which it becomes an integral part. Azoic dyes contain the azo group (and formic acid, caustic soda metallic compounds, and sodium nitrate); especially for application of cotton. While textile mills predominantly use them, azo dyes can also be found in the food, pharmaceutical, paper and printing, leather, and cosmetics industries.

Dye removal techniques:

There are many methods available for the treatment of azo dyes and of dye containing wastewater. Various physical, chemical and biological pre and post treatments can be employed to remove colour from dye containing wastewater. Physical treatment includes membrane filtration, coagulation, flocculation, precipitation, flotation, adsorption, ion exchange, ultra sonic mineralisation and electrochemical treatments. Chemical technology includes oxidation (chlorination, bleaching, ozonation) advanced oxidation (Fenton's, photo oxidation) and reduction. Biological techniques include bacterial, fungal and algal biosorption and biodegradation in aerobic, anaerobic, anoxic or combined anaerobic/ aerobic treatment processes.

Fungi:

Fungi thrive well in inhospitable habitats with environmental extremes because of their enzyme system (Cooke, 1979). Fungi are involved in the biodegradation of undesirable materials or compounds and convert them into harmless, tolerable or useful products. Fungi are recognized for their superior aptitudes to produce a large variety of extracellular roteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints (Lilly and Barnett, 1951; Cochrane, 1958). Fungal systems appear to be most appropriate in the treatment of coloured and metallic effluents (Ezeronye and Okerentugba, 1999).

Fungi not only produce various metabolites like citric acid, homogeneous proteins, heterogeneous proteins, peroxidases but have shown their effectiveness for removal, reduction and detoxification of industrial effluents ingredients. Bioremediation refers to the productive use of microorganisms to remove or detoxify pollutants, usually as contaminants of soil, water or sediments that otherwise threaten public health. Microorganisms have been used to remove organic matter and toxic chemicals from domestic and industrial waste discharged for many years (Gupta and Mukerji, 2001).

Saccharomyces cerevisiae :

The decolourising capabilities of *S. cerevisiae* towards several azo dyes were compared. The presence of dyes and degradation products in the growth medium did not affect nor growth nor viability of cells. The specific degradation rates obtained showed that *S. cerevisiae* is much more efficient in the decolourisation of dyes.

In the previous reports use of the ascomycete yeast, *Candida zeylanoides* isolated from contaminated soil to reduce model azo dyes (Martins *et al*., 1999). The characterisation of an enzymatic activity is described in further studies with the yeast, *Issatchenkia occidentalis* (Ramalho *et al.,* 2004), and the enzymatic system involved is presented in a work with *Saccharomyces cerevisiae* (Ramalho *et al.,* 2004).

So, it was important to find out an easy cost effective biological treatment of dye effluent. In the present study, we have studied the yeast mediated degradation of azo dye molecules taking methyl orange as model dye compound.

Following experimental parameters were studied to get maximum degradation/oxidation of the dye molecule:

Effect of pH, yeast concentration, dye concentration, inorganic anions on degradation, kinetics of photo degradation different initial dye concentrations, degradation of dye molecules were monitored by UV-visible spectrometer, high pressure liquid chromatography (HPLC), LC-MS, NMR and measurement of total organics in terms of chemical oxygen demand (COD). The toxicity of degraded sample measured using cytotoxicity experiment.

EXPERIMENTAL METHODOLOGY

Degradation and decolourisation of methyl orange:

Materials:

The materials used in the study : Methyl orange was received from S.D. Fine-chem (Mumbai) and sodium bicarbonate (Purified, E.Merck, Mumbai), potassium dichromate (Qualigens, Mumbai), silver sulphate, mercury sulphate, sulphuric acid, hydrochloric acid, sodium hydroxide, sodium sulphate, sodium nitrate, sodium carbonate (Purified, E.Merck, Mumbai). All the reagents were of by double distilled and ultra pure water. The commercially available baker 's yeast granule, *Saccharomyces cerevisiae* was dissolved in sterile distilled water.

Experimental procedure:

Effect of pH on degradation:

The effect of pH experiment was conducted to determine the pH where in the maximum extend of degradation would take place in the dye and the corresponding pH was chosen for the further experiments.

The pH meter (Elico pH meter, Hyderabad, India) was calibrated using buffer solutions.

The solutions with varying pH were prepared form the neutral solution using sodium hydroxide for the basic range and hydrochloric acid for the acidic range. The different pH's prepared from 1 to 10.

The dye of concentration from 200 ppm prepared from 1000 ppm of dye solutions. To study the effect of pH on degradation, 50 ml at the dye concentration 200 ppm with respective pH solutions and constant weight of yeast. These experiments were also repeated with air flow. The initial and final optical densities of the samples were measured using the spectrophotometer at respective λmax wavelength of the dye molecules.

Effect of dye concentration:

This experiment was conducted to estimate the dye concentration that would exhibit a greater extend of degradation with constant amount of yeast. This experiment was also conducted with air flow. The dye concentrations were varied from 5 to 1000 ppm. The final concentration of dye was measured using spectrophotometer.

Effect of concentration of yeast:

This experiment was conducted to estimate the catalyst concentration that would exhibit a greater extend of degradation of dye with constant concentration of dye .These experiment also conducted with air flow. The catalyst concentrations were varied from 1 to 20 mg. The final concentration of dye was measured using spectrophotometer.

Effect of electrolyte concentration:

In order to obtain the optimum concentration of the electrolyte that enhanced the degradation of the dye of 200 ppm concentration, this experiment was conducted with a total sample volume of 50 ml. The electrolytes sodium chloride, sodium sulphate, sodium carbonate, potassium sulphate were added to the 200 ppm dye samples in varying percentages of electrolytes from 0 to 15mg.

Analysis of dye melocule by UV-visible spectrophotometer:

Absorbance measurements were used to monitor the degradation of the dyes at the λmax of the dyes by scanning the dye using a range of 400-800nm (for example, methyl orange at 465nm, respectively). Standard dye solution at the concentrations of 0.1 to 100 ppm was prepared, and their absorbance was measured at the λmax wavelength of the dye solutions using UV-Visible spectrophotometer (Shimadzu Japan, 2101 PC). The calibration graph was prepared for the dye concentration varying from 0.1 to 100 μM.

High pressure liqiud chromatography:

hplc was performed using the mobile phase acetronitrile : water in the ratio 60:40 with the help of uv detector and the flow rate was 0.5ml/min. The column used was c-18 column. The sample was run for about fifteen minutes.

Liquid chromatography- mass spectroscopy:

lc-ms was performed using the mobile phase 10mm ammonium acetate in water : methanol. The flow rate of the sample was 1.2ml/min. The column used was zorban xdb c-18 column. The chromatogram was run for nearly ten minutes.

Nuclear magnetic resonance spectroscopy:

1D NMR was performed for the parent dye methyl orange and the completely degraded sample. The structure of the degraded product can be found further using 2D NMR.

Fourier transform – infrared:

The parent dye and the degraded product formed at the temperature of 28^{0}C were run using FTIR to find the functional groups present in the parent dye methyl orange and in the degraded product.

Chemical oxygen demand (cod) by closed reflux tritrimetric method:

The tubes were placed in the thermoreactor spectroquant TR-320 (Merck, Germany) block digester preheated to 148^{0}C and the refluxed for 2 hour behind a protective shield. After being refluxed the vessels were cooled to room temperature were in some mercuric sulfate may precipitate out but will not affect the analysis. The samples were transferred to a conical flask for titration. 0.05 to 0.10 ml of ferrion indicator was added and rapidly stirred when it was titrated against standardized 0.05 M FAS. The end point is the sharp colour change from blue-green to reddish brown, although the blue-green may reappear in few minutes. In the same manner a blank containing the reagents and a volume of distilled water equal to that of the sample was refluxed and titrated.

$$\text{COD as mg } O_2/L = \frac{(A-B) \times M \times 1000}{\text{ml of sample}}$$

where

A = ml FAS used for the blank.

B = ml FAS used for the sample.

M = molarity of FAS and

= milli equivalents weight of oxygen x 1000 ml/l.

EXPERIMENTAL FINDINGS AND DISCUSSION

The experimental findings of the present study have been presented in the following sub heads:

Cytotoxicity test: Brine shrimp assay:

Acute toxicity test on brine shrimp:

As the method was going to be utilized for the water purification, specific precaution was taken. A basic toxicity test was conducted on brine shrimp (artemia). This test reveals the possibilities of this sample as an coagulating agent without any toxicity. Many researchers had failed to perform this test but our intension was to confirm that the degraded sample is not toxic to the environment. Concentration of 200 ppm was considered as the safest dosage where the artemia was surviving.

Concentration - 200 ppm, Live artemia, Dead artemia

Control - 10

Parent dye - 3 7

Degraded sample at 28^0 C 9 1

Degraded sample at 37^0 C 8 2

The above mentioned results were very clearly giving the information about the non-toxic nature of the degraded sample, when compared to the parent dye. The artemia survived well in the degraded sample proving the parent dye methyl orange which was degraded and was less toxic to the environment.

In this project, we have proposed a novel method of high research significance and actual application for yeast mediated azo dye degradation which is cost effective and environmentally friendly novel method. The use of yeast as a catalyst to degradation organic pollutants in water (in this experiment methyl orange dye was selected). The experimental study and mechanism analysis led to the following conclusions :

– The efficiency of degradation was high in alkaline pH.

- Yeast favours degradation of methyl orange.
- Cost of yeast has apparent effect on catalyst effect and optimum cost is about 125 mg per 100 ml.
- The more accurate optimum cost needs to be determined by further investigation.
- Salinity can apparently affect the degradation effect of yeast. The higher the salinity, the better the degradation by yeast.

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